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Chapter

Natural Products Altering GABAergic Transmission

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Abstract

Gamma-amino butyric acid (GABA) is a major inhibitory neurotransmitter found in several regions of the brain and known to have various significant physiological roles as a potent bioactive compound. Malfunction of GABAergic neuronal signaling prompts to cause severe psychiatric symptoms in numerous mental disorders. Several drugs are available in clinical practice for neuropsychiatric disorders targeting through GABAergic pathway, with notable adverse effects. Interestingly, in recent years, researchers are focusing on natural compounds altering GABAergic neurotransmission for various psychiatric disorders due to its wide range of therapeutic efficacy and safety. The enormous variety of natural compounds, namely alkaloids, flavonoids, terpenoids, polyacetylenic alcohols, alkanes and fatty acids were reported to alter the GABAergic transmission through its receptors and or by influencing the transmission, synthesis and metabolism of GABA. Natural compounds are able to cross the blood brain barrier and influence the GABA functions in order to treat anxiety, mania, schizophrenia and cognitive disorders. Therefore, this current chapter describes on natural products which have the potential to alter the GABAergic neurotransmission and its therapeutical benefits in treating several neuropsychiatry disorders using various pharmacological methods.

Keywords: Natural products, GABA, agonist, metabolism, allosteric modulation, psychiatric disorders

1. Introduction

The ground breaking discovery of Gamma-aminobutyric acid (GABA) played an astonishing role in neural control theory in 1950's. In the human cortex GABA is the primary inhibitory neurotransmitter [1]. In the initial developmental stage of life, GABA functions as an excitatory element which influences many physiological processes like neuronal proliferation, neurogenesis, migration, differentiation and preliminary circuit building. After maturation of CNS, GABA acts as an inhibitory neurotransmitter which is controlled as chloride or cation transporter expression. GABA also plays a vital role in interstitial neurons development of white matter along with oligodendrocyte development. Whereas the basic fundamental cellular mechanisms are not well described though it is proven that a lot of neurological diseases are well involved through GABA dependant pathway which includes white matter abnormalities, including anoxic-ischemic injury, anxiety, insomnia and schizophrenia [2]. GABA receptors are majorly classified into two main types ionotropic $GABA_A$ and

GABA$_C$ receptors and the metabotropic GABA$_B$ receptor. GABA$_A$ acts by activating the fast-hyperpolarizing negative ion channel (Cl$^-$) and diffuse by the means of concentration gradient to hyperpolarize post synaptic mature neurons [3, 4]. Whereas another kind of ionotropic receptor was discovered GABA$_C$ with 3ρ subunits [5]. GABA$_B$ receptors consist of two subunits, GABA$_{B1}$ and GABA$_{B2}$ which are responsible for slower inhibitory transmission. These receptor activations are coupled with K$^+$/Ca$^+$ channels through G-protein mediated secondary pathway [6].

Natural molecules with a wide range of chemical structures have been shown to have GABA$_A$ receptor modulating potential due to the structural heterogeneity of and more than one number of binding sites. It has different pharmacological effects depending on the mechanism of action, the binding site and the affinity of the compounds. These effects have been investigated using different *in vitro* and *in vivo* models [7–9].

The versatile binding nature of benzodiazepine binding site of GABA receptor allows multiple molecules to bind and modulate the functions of GABA in a very specific manner. So, this class of compounds are used for the treatment of anxiety, convulsion, insomnia by non-specifically modulating all five α subunits. This non selective nature of these compounds generates unwanted side effects like tolerance and dependence. Therefore, there is an immediate need for finding safe drugs, with increased anxiolytic and decreased sedative potential. In recent decades, various reports have been made on natural products with GABAergic activity and, different various methods have been used to describe the effects. Hence, this review aimed to collect the existing data and make the obtained results as comparable as possible, thus facilitating the discussion of structure–activity relationships [10].

1.1 Synthesis

GABA is mainly produced from α-decarboxylation of glutamate by the enzyme glutamic acid decarboxylase (GAD) and metabolized by the actions of GABA-transaminase (GABA-T) and succinic semialdehyde dehydrogenase (SSADH) into succinate respectively. Through the use of the pyridoxal-5′-phosphate-dependent interconversion steady state concentration of GABA is achieved in-vivo (apo-GAD). At least 50 percent of the total GAD present in the brain is apo-GAD [10]. Inorganic phosphate promotes the activation of GAD and blocked by aspartate, GABA and ATP. The ATP facilitates and stabilizes apo-GAD formation which further stimulates the development of GABA. At 37°c temperature apo-GAD has a half-life of few minutes without ATP. GAD mainly consists of two isoforms of distinct molecular weights (65 and 67 kD) which are the products of chromosomes 2 and 10 in humans.

After synthesis, GABA vesicular release has specific mechanisms. GABA is assembled using Mg$_2$$^+$ activated ATPase into vesicles. This method is energy-dependent and requires adenosine triphosphate and magnesium. Calcium-dependent GABA vesicular release appears to result in a temporary increase in the synaptic cleft's GABA concentration and the binding of the receptor to evoke action. Through the sodium and chloride reuptake mechanism of the GABA transporter (GAT) to the presynaptic neuron and surrounding glia, quick synapse removal takes place. GABA is then reused into metabolites that are eventually used for GABA resynthesis by breakdown. GABA-oxoglutarate transaminase, succinic semial-dehyde dehydrogenase and glutamate decarboxylase (GAD) are three enzymes required for GABA metabolism and resynthesis. The deterioration of GABA to succine semi-aldehyde is catalyzed by the enzyme GABA oxoglutarate transaminase. The latter is then oxidized by means of succinic semialdehyde dehydrogenase into succinic acid. Ligands associated with these GABA procedures will regulate the action of GABA [11].

2. GABA receptor physiology and GABA ligands

In 1981, $GABA_A$ & $GABA_B$ subtypes of GABA were discovered by Hill and Bowery. $GABA_A$ was reported as chlorine sensitive ion channel which is allosterically modulated by barbiturates, benzodiazepines, neurosteroids and ethanol. Along with this $GABA_B$ receptors couple with Ca^+ and K^+ channels via G protein second messenger system. This receptor activation specifically happens through baclofen which is resistant through $GABA_A$ modulators [12].

As $GABA_B$ receptors are dimeric metabotropic in nature and the structure of pentameric $GABA_A$ receptors ideal for allosteric regulation. So research on these receptors is likely to develop novel therapy for the treatment of neurological and psychological disorders [13, 14] (**Figure 1**).

2.1 $GABA_A$ receptor

Among the three types of GABA receptors, the $GABA_A$ receptor is the best characterized one. For several selective ligands, this channel has numerous binding sites. One class of therapeutic drugs linked to this target are receptor modulators: benzodiazepines, non-benzodiazepines and barbiturates, most of which improve the effect of GABA by increasing the chloride channel opening [13].
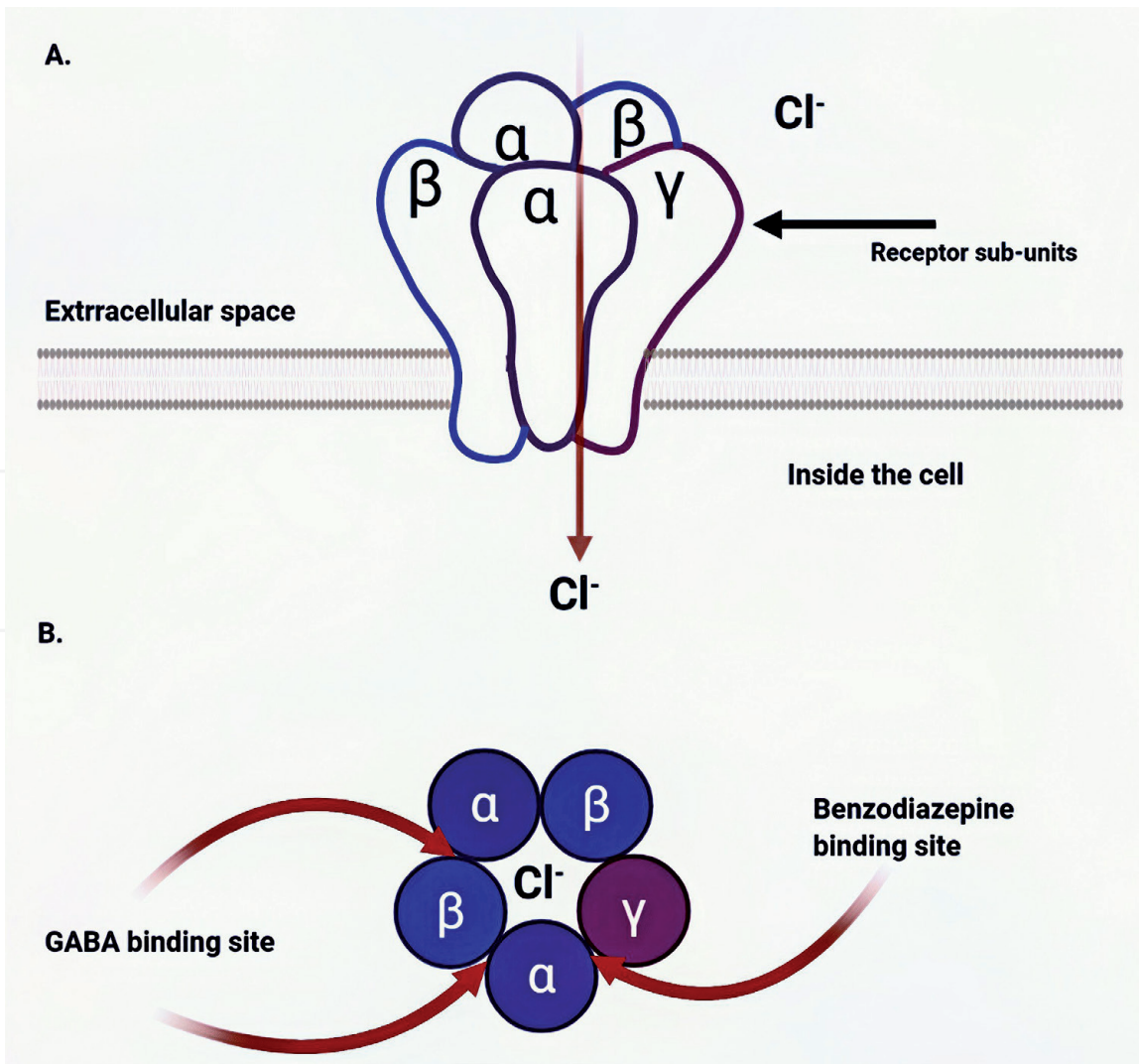
Figure 1.
A. The subunits of $GABA_A$ receptor and chlorine channel. B. Represents the barbiturate and benzodiazepine binding sites on GABA receptor subunits.

The GABA$_A$ agonist, muscimol, antagonist bicuculline and picrotoxin and inverse agonist FG 7142 are additional ligands which bind to the GABA$_A$ receptor. Some of these agents do not seem to have therapeutic benefits, but when used as pharmacological tools for the GABA$_A$ receptor they are the most significant ligands. Neuro-active steroids and partial benzodiazepine agonists (PBAs) are some newly discovered agents which are coming into recent considerations [14]. PBAs (e.g., bretazenil, imidazenil) are GABA$_A$ receptor activators, similar to benzodiazepines. Although they tend to have lower effectiveness compared to full benzodiazepine agonists, they give a more favorable side effect profile. Compared to other configurations found in more selective areas, this subtype is common throughout the brain [15].

2.2 GABA$_B$ receptor

GABA$_B$ receptors have seven membrane-spanning amino acid domains which are connected by a G-protein to its signaling pathway (K$^+$, Ca^{++} ion channels or adenylate cyclase). Presynaptic GABA$_B$ receptors are majorly coupled to calcium channels and their stimulation by the receptor results in decrease of calcium conductance and decline of GABA release. Thus, the receptors auto-regulates the discharge of GABA$_A$ and gives the GABA$_A$ system with negative feedback. On the other hand, Post-synaptic GABA$_B$ receptors are primarily linked to potassium channels and their stimulation led to increased conductance of K$^+$, hyperpolarization and decreased excitability of the neurons. The opening of T-type calcium channel is mainly associated with the actuation of GABA$_B$ receptor, resulting in calcium spiking activity that can contribute to absence seizure and is also included in signaling through the pathway of adenylate cyclase. It is therefore assumed that mediation of the GABA$_B$ receptor occurs through at least two distinct subtypes receptor [16–18].

2.3 GABA$_C$ receptor

The GABA$_C$ receptor, a subtype of GABA$_A$ receptor characterization started when the analogue of GABA cis-4-aminocrotonic acid (CACA) in cat spinal interneurons developed a depressant action, which was not inhibited by the GABA$_A$ antagonist bicuculline and varied from the depressant actions of the GABA$_B$ agonist baclofen. The GABA$_C$ receptor is distinguished from both GABA$_A$ and GABA$_B$ by their pharmacological actions. GABA$_C$ is structurally different from GABA$_A$ because GABA$_C$ is hetero oligomeric and homo oligomeric which means it composed of many subunits of the same subtype, it can be either r1 or r2 [19].

3. GABAergic system and neurological disorders

The main components of brain inhibitory circuits are networks of (GABAergic) interneurons in the amygdala [20]. This neurotransmitter is essential to maintain a balance between neuronal excitation and inhibition. Both glutamatergic neurons and the GABAergic interneurons compose of the basolateral nucleus (BLA). A relatively small group of GABAergic inhibitory neurons is closely regulated by Glutamatergic neurons. Devastation of GABAergic BLA inhibition, such as anxiety and depression, emotional dysregulation, and seizure actions, can cause hyperexcitability of the The central amygdala (CeA) consisting only of GABAergic neurons acts by converging inputs from the BLA as the primary output nucleus of the amygdala. In addition, the BLA, the central amygdala and all their associations play a key role in the regulation of the GABAergic system. As a result, these

GABAergic amygdala neurons are properly trained to perform a central role in the stress management. Nonetheless, even less is known about the association between the GABAergic amygdala inhibitory system and stress [21].

The sedative and hypnotic effects are mediated by α_1 subunit of $GABA_A$ receptors, whereas the anxiolytic effect is exhibited by the positive regulation of α_2 and (or) α_3 subunit of $GABA_A$ receptors. Furthermore, in learning and memory, the α_5 subunits play an important role. The cause of side effects, such as muscle relaxation or anterograde amnesia, is because of benzodiazepines, which are widely used in the treatment of anxiety, insomnia and seizures, functioning on various subunits (α_1, α_2, α_3 and α_5). Such drawbacks include the growth of resistance and dependence. The development of new and safer drugs with, for example, an efficient anxiolytic yet low sedative potential is therefore urgently warranted. Various studies have recently been performed on natural products with GABAergic involvement, and various types of approaches have been used to clarify the findings. Consequently, the purpose of this analysis is to gather current evidence and generate the findings obtained, thereby promoting the discussion of structure activity relationships [9].

In knock-out mice special kind of $GABA_B$ receptors are being introduced in mice that lack subunits of the $GABA_B$ receptor. In addition to psychiatric conditions, the phenotype of these mice shows evidence of $GABA_B$ receptor activation in epilepsy, sensorimotor gating, nociception and temperature control [22]. With almost the same behavioral phenotype as $GABA_{B1}$ Knockout mice whereas mice that lack the $GABA_{B2}$ subunit are currently developed. Some data suggest that these phenotypes underlie the lack of heteromeric $GABA_{B1}$ and $GABA_{B2}$ receptors. In order to evaluate the anxiolytic ability of other positive $GABA_B$ receptor modulators, further studies are required, but current evidence suggests that they may be a new category of anxiolytics with a higher side effect profile than benzodiazepines. The mechanisms involved in the anxiety activity impact of $GABA_B$ receptors are not well known. Future research should also focus on behavioral and electrophysiological approaches to the activation of $GABA_B$ receptors in major anxiety-related brain regions [23].

3.1 GABAergic system in schizophrenia

In late adolescence or early adulthood, schizophrenia is a mental health condition that commonly occurs. Its impact on speech, thinking, emotions and other areas of life can affect the social interactions and daily activities of people. In the presynaptic neuron, the carrier protein is available in GAT-1 and is mainly responsible for GABA reuptake in synapse. It plays a significant role in both phasic and tonic inhibition which is regulated by GABA. The synaptic potential of GABA is terminated by GAT-1 and it is managed by the duration and adequacy of GABAergic neurotransmission therefore, decreased GAT-1 levels demonstrate enhanced accessibility of GABA. In schizophrenia, numerous studies show decreased levels of mRNA encoding for the GAT-1 protein along with the decreased expression of GAD 67 mRNA. GAT-1 mRNA delivery is decreased and generally unchanged in most GABAergic neurons. GAT-1 mRNA concentration fluctuations are recognized in chandelier neurons. In schizophrenia, the thickness of immunoreactive GAT-1 cartridges is reduced, although axon terminal marker in other populations remains unaltered. Relatively low GAT-1 immunoreactive cartridge thickness indicates a significantly reduced GAT-1 protein correlated with a reduced level of GAT-1 mRNA. Therefore, in individuals with schizophrenia, the amount of GAT-1 protein-enclosing chandelier neurons decreased whereas the number of neurons comprising parvalbumin remained consistent. This outcome infers that the decreased degrees of GAT-1 mRNA are restricted to chandelier neurons.

The decline of GAD67 mRNA coding in the prefrontal dorsolateral cortex is the most predictable post-mortem finding in schizophrenia, which led to decrease in GAD67 levels of protein, despite the fact that this has been less widely considered. The schizophrenia-influenced subset tends to incorporate GABAergic neurons comprising parvalbumin. Expression of parvalbumin mRNA in schizophrenia is diminished in layer 3 and 4 of the prefrontal cortex (PFC). In the prefrontal cortex (PFC), the recent discovery indicates that the decreased articulation of GAD67 mRNA is unique for the GABA neuron subgroup [24].

Adequate histopathological data also suggests that, the association of GABAergic neurotransmission impairment with pathologies and cognitive dysfunctions of schizophrenia. The primary motor cortex (PMC), primary visual cortex (VC), anterior cingulate cortex (ACC) is distinguished by the similar GABAergic gene expression deficits as shown in the Dorsolateral prefrontal cortex, which includes selective parvalbumin-containing GABA neuron involvement. The greatest decreases in mRNA encoding levels for parvalbumin have been reported. In serious case reduction in the α_1 and δ subunits of GABA receptors, GAD67 mRNA, GAD65 mRNA and GAT-1 mRNA is displayed in the brain regions [10, 25].

3.2 GABAergic system in anxiety and depression

Both in animals and humans, depression and anxiety are most frequent causes of persistent stress. Two mechanisms are defined by anxiety models: fear processes are believed to be developed to allow us to change our emphasis on the first hint of risk and behavioral modification in order to prevent or eliminate an imminent or predicted overt danger [26].

The long-term potential activity strongly depends on the augmentation of GABA signaling which process through the $GABA_A$ receptors namely α_1 and α_2. The long-term potential response triggers are not only restricted to $GABA_A$ but also to the $GABA_B$ receptor. The $GABA_B$ receptor antagonists causing the long-term potential response on cortical along with thalamic centripetal synapses whereas the thalamic feed needs postsynaptic response from NMDA-receptor. The cortical actions controlled by pre-synaptic response on increased glutamate response by NMDA receptor independent activity, so activating GABA synapse thereby inducing $GABA_B$ receptor might help to arrest non associated long-term potential there by reducing agitation response [27].

By protruding to the central amygdala (CEA), CEA output neurons control the GABAergetic tone and form a spontaneous active neuron in lateral subdivisions. Aversive stimulus can reduce this inhibitory tone. CEA consists primarily of localized GABA neurons and the inhibition of GABA occurs through $GABA_A$ α_2 receptor. Therefore, for benzodiazepine-induced anxiolysis and anti-panic activity, CEA considered to be a significant target [28].

3.3 Epilepsy and GABAergic system

Epilepsy can be the consequence of disturbances in the homeostasis involving other neurotransmitters and neuromodulators, for example, glutamate, adenosine, norepinephrine, and acetylcholine. GABA receptor or transporter function alteration can allow the occurrence of seizure in the presence of normal GABA levels. Some data indicates that low occipital lobe GABA concentration (remote from the seizure focus) is a risk factor for seizure recurrence. Low GABA levels predispose but may not be sufficient for seizures to become clinically effective [28, 29].

In case of adults, status epilepticus induces a complete re-organization of the networks, with cell death, axonal growth leading to an increased glutamatergic

drive. This, in turn, will decrease the threshold of seizure generation and thus contribute to seizure generation. Somatostatin innervates the dendrites of the principal cells in the hippocampus and triggers a chemical imbalance between excitatory and inhibitory neurotransmitters which leads to a reduction of the inhibitory strength that is necessary but not sufficient to generate ongoing seizures. An additional important factor is the persistent increase of the intracellular chloride concentration that leads to a long-lasting shift in the depolarizing direction of the actions of GABA that will also contribute to seizure generation [30, 31].

4. Natural products and GABA

Due to the different binding sites present on GABA (A) receptor, various receptor modulating compounds have been identified and depending on the mode of action, the affected binding site, and the compounds' affinity. Radioligand binding assays have been confirmed the capacity of the ligand for the displacement of a molecule from its binding site. Various studies helped us understand the link between modulation of the receptor and associated effects, such as anxiolytic, sedative, and anticonvulsive properties (**Figure 2**; **Table 1**).

Radioligand binding assays are simple but influential tool for reviewing receptors. They mainly analyze the interactions of hormones, neurotransmitters, growth factors, and related drugs with the receptors, studies of receptor interactions with second messenger systems, along with the characterization of regulatory changes in receptor number, subcellular distribution, and physiological function. So these assays are widely used in a numerous disciplines, including pharmacology, physiology, biochemistry, immunology, and cell biology. The fundamentals of the radioligand binding assay are fairly simple. The receptor of interest is incubated with an appropriate radioligand for a suitable period of time and then the radioactivity bound to the receptor is determined. There are three major types of experiments:
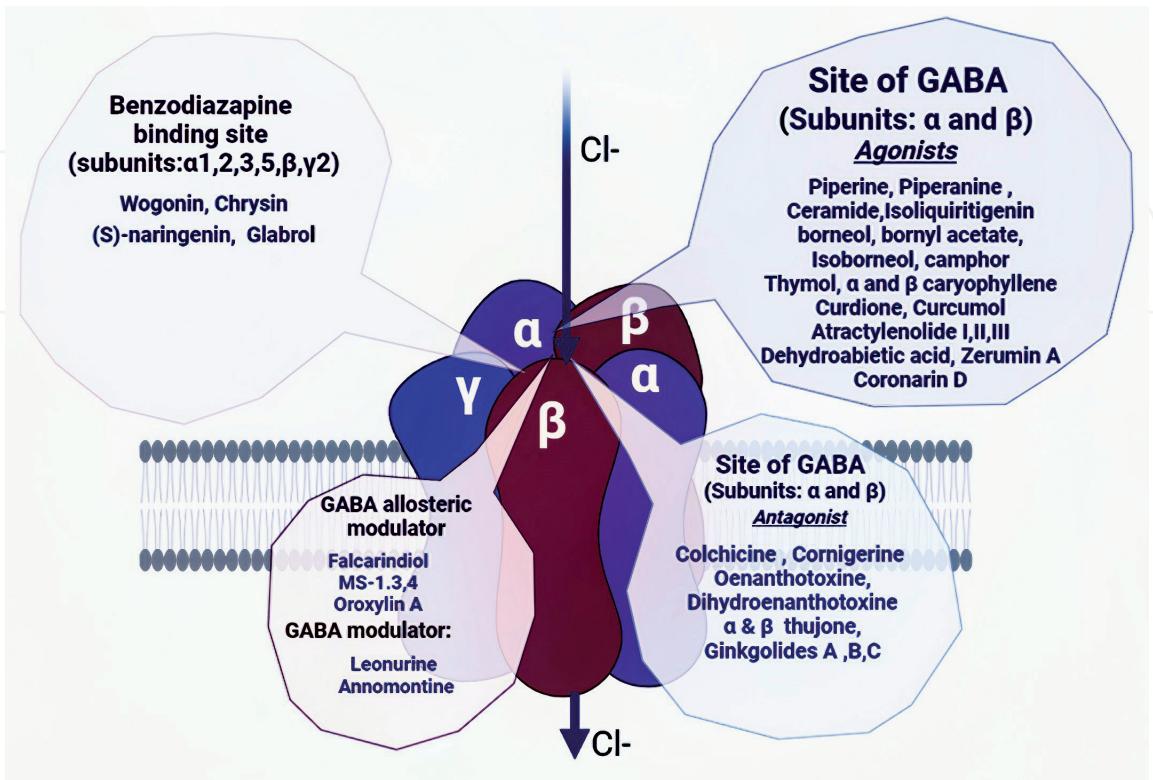

Figure 2.
Action of natural products on the specific site of GABA receptor.

Class of Compound	Source	Mechanism	Active Componud	In-Vivo/In-Vitro	Reference
Alkaloids					
	Colchicum autumnale, Colchicum szovitsii	GABA competitive antagonist	Colchicine Cornigerine	Electrophysiological studies, Radio ligand binding assay	[32, 33]
	Leonurus japonicus	GABA modulator	Leonurine	Radio ligand binding assay	[34]
	Piper nigrum	GABA (A) receptor agonist	Piperine piperanine	Electrophysiological studies	[35]
	Annona purpurea	GABA modulator	Annomontine	Elevated plus maze test	[36]
	Aconitum leucostomum	non-competitive GABA(A) receptor antagonist	Songorine	Electrophysiological studies, Radio ligand binding assay	[37]
Alkanes					
	Sarcophytum auritum	GABA agonist	Ceramide	Elevated plus maze test & Light dark test	[38]
	Oenanthe fistulosa	GABA (A) receptor antagonist	Oenanthotoxine, Dihydroenanthotoxine	Electrophysiological study	[39]
	Oenanthe crocata	GABA (A) allosteric modulator	Falcarindiol	Electrophysiological study	[40]
	Cussonia zimmermannii	GABA (A) allosteric modulator	MS-1 MS-2 MS-4	Electrophysiological studies	[41]
Flavones					
	Scutellaria baicalensis	Allosterically blocks GABA-mediated receptor	Oroxylin A	Electrophysiological study, Radio ligand binding assay	[42]
	Scutellaria baicalensis	Acts at benzodiazepine site of GABAA receptor	Wogonin	Radio ligand binding assay, Elevated plus maze test and hole board test	[43]
	Scutellaria baicalensis	Partial agonist of central benzodiazepine receptor	Chrysin	Elevated plus maze and hole board test	[44]

Class of Compound	Source	Mechanism	Active Componud	In-Vivo/ In-Vitro	Reference
Flavanes					
	Mentha aquatic	Acts at benzodiazepine site of GABA receptor	(S)-naringenin	Radio ligand binding assay	[45]
	Glycyrrhiza glabra	Acts at benzodiazepine site of GABA receptor	Glabrol	Radio ligand binding assay	[46]
Isoflavanoids and chalcones					
	Adenocarpus cinncinatus	GABA (A) receptor agonist	2′,4′,7-trihydroxy-8-(3-methylbut-2-en-1-yl) isoflavone	Electrophysiology study	[47]
	Angelica dahurica	GABA (A) receptor agonist	Isoliquiritigenin	Electrophysiology study, Radio ligand binding assay	[48]
Monoterpenes					
	Valeriana officinalis, Matricaria chamomilla, Lavandula officinalis	GABA (A) receptor agonist	(+)borneol, (−)-borneol, (−)-bornyl acetate, Isoborneol, camphor	Electrophysiology study	[49]
	Artemisia absinthium	GABA (A) receptor antagonist	α- thujone, β- thujone	Electrophysiology study; Radio ligand binding assays	[50]
	Thymus vulgaris	GABA (A) receptor agonist	Thymol	Electrophysiology study	[51]
Sesquiterpenes					
	Sideritis hyssopifolia	GABA (A) receptor agonist	α caryophyllene, β caryophyllene	Electrophysiology study	[52]
	Rhizoma curcumae oil	GABA (A) receptor agonist	Curdione, Curcumol	Electrophysiology study	[53]

Class of Compound	Source	Mechanism	Active Componud	In-Vivo/ In-Vitro	Reference
	Atractylodes macrocephala	GABA (A) receptor agonist	Atractylenolide I Atractylenolide II Atractylenolide III	Electrophysiology study	[47, 54]
	Illicium anisatum	non-competitive antagonist of GABA$_A$ receptor	Anisatin	Electrophysiology study	[55]
Diterpenes					
	Boswellia serrata	GABA (A) receptor agonist	Dehydroabietic acid	Electrophysiology study	[47]
	Curcuma kwangsiensis	GABA (A) receptor agonist	Zerumin A Coronarin D	Electrophysiology study	[56]
	Ginkgo biloba	GABA (A) receptor antagonist	Ginkgolides A Ginkgolides B Ginkgolides C	Electrophysiology study	[57, 58]
Triterpenes					
	Actaea racemosa	GABA (A) receptor agonist	Cimigenol-3-O-β-D-xylopyranoside 25-O-acetylcimigenol-3-O-α-L-arabinopyranoside	Electrophysiology study	[59]
	A. racemosa	GABA (A) receptor agonist	23-O-acetylshengmanol-3-O-β-D-xylopyranoside	Electrophysiology study, Elevated Plus maze test and open field test	[59, 60]

Table 1.
Natural compounds altering GABAergic transmission.

saturation, kinetic, and inhibition. A saturation curve can be made by considering amount of receptor as constant and concentration of radioligand as variable. From this type of experiment the receptor density and the affinity of the receptor for the radioligand can be estimated. If the amount of receptor and radioligand is constant and the time is the variable, then kinetic data which are obtained from forward and reverse rate constants can be assessed. If the amount of a competing nonradioactive drug included in the incubation is the only variable, then the affinity (Ki) of that drug for the receptor identified by the radioligand can be estimated [61].

In Xenopus oocytes assay, xenopus oocytes are the immature egg cells of the South African clawed frog *Xenopus laevis*, these have striking appearance with two colors, the light colored vegetal pole and the dark animal pole, where the nucleus is found. TEVC recording (This method is a type of patch-clamp electrophysiology method to inject current into a cell with one electrode and recording the change in voltage with the other electrode) is used to measure membrane potential of the oocyte is clamped at a constant value. Two electrodes spear the oocytes in which one intracellular microelectrode measures the membrane potential (voltage electrode) and the second one (intracellular microelectrode) controls the current. This is also called current electrode and uses as a feedback circuit to pass sufficient current to the oocyte for maintaining the voltage clamp. The current which is flowing through the current electrode can provide a measurement of the of chloride specific ion channels [62].

4.1 Alkaloids

Radioligand binding assay using [^{35}S] TBPS and [^{3}H] flunitrazepam analyzed the weak partial agonistic activity of Colchicine and (−) cornigerine along with six other colchicinoids from *Colchicum brachyphyllum*. These two molecules displayed 25% of the action of 10 μM allopregnanolone, but the (−) activity of colchicines was none. (−) colchicines acted as a GABA competitive antagonis [32, 33]. GABA modulation is also recorded in proto alkaloid leonurine, belonging to an East Asian herb called *Leonurus japonicus*, indicated for anxiety, depression, nervousness, and insomnia. The molecule showed half-maximal inhibitory concentration (IC$_{50}$) values of 15 μg/mL and 123 μg/mL, respectively in a radio ligand assay with [^{3}H] gabazine and [^{3}H] flumazenil [34].

Piperine and piperanine belonging to the class of piperidine-alkaloids were investigated in the immature egg cell of *Xenopus laevis*. The binding site of the molecule was confirmed to be a benzodiazepine binding site as Flumazenil (5 mg/kg) [35].

A β-carboline named annomontine also shows GABA dependant activity which was separated from the plant *Annona purpurea* [36]. In the EPM test, the compound increased the time spent in the open arms and the open arm entries at 10 and 30 mg/kg, but not the total arm entries. These effects are controlled via the benzodiazepine binding site as it was confirmed with antagonist Flumazenil at a dose of 3 mg/kg. Another alkaloid, a non-competitive GABA(A) receptor antagonist is diterpene alkaloid and this was separated from the plant *Aconitum leucostomum* having an IC$_{50}$ 19.6 μM. Radioligand studies with the help of [^{3}H] muscimol produced an IC$_{50}$ value of 7.06 μM and a Kd value of 6.31 nM. Specific binding site specificity was shown by β-carbolines and picroacridine alkaloids as they bind to the benzodiazepine binding site Benzodiazepine binding sites can at least be excluded for piperidine alkaloids and protoberberine alkaloids. This holds for songorine in GABA/muscimol binding site as well [37].

Three colchicinoids displayed unspecific binding with weak action on both benzodiazepine and TBPS/bicuculline binding sites. Colchicine is the antagonist, but androbiphenyline and cornigerine are partial agonists. Protoalkaloid Leonurine

shows binding to various sites, with decreased affinities to, GABA/muscimol and the benzodiazepine binding site. Protoberberine type 2 alkaloids were able to modulate GABA(A) receptors, but unsaturated type 1 alkaloids displayed no effects.

4.2 Alkanes

The odor substance, 1-octen-3-ol is part of the GABA$_A$ sensory receptor modification research and has a stimulation rate of 295 ± 50 percent at a particular concentration of 300 μM and 1 μM GABA [52]. Ceramide (N-[(2S,3R,4E,6E)-1,3-dihydroxyhenicose-4,6-dien-2-yl] tridecanamide) separated from the Red Sea soft coral *Sarcophytum auritum*. This works as GABA agonist and produce anxiolytic effect in animal models evaluated by EPM test in which the animal spent more time in open arms and time spent in light in light/dark test whereas all its action can be altered by the action of bicuculline (GABA antagonist) [38, 52].

Two polyacetylenes extracted from *Oenanthe fistulosa*, Oenanthotoxine and dihydroenanthotoxine, provided major inhibitory activity on GABA receptors with IC50 values of 0.835 μM and 1.29 μM respectively on GABA (A) receptors The potential explanation for the indications which include water drop worm intoxication (Oenanthe crocata) and facial muscle contractions which is due to the inhibition of GABAergic reactions. The substance that allosterically regulate GABA-binding, non-competitively inhibits ion channel and also eradicates the desensitization of the receptor was constituted by modes of action of oenanthotoxin [39].

At a very low concentration the component falcarindiol obtained from *Oenanthe crocata* effectively regulates GABAergic currents. This component at low concentration (1 μM for falcarindiol and 0.1 μM for falcarinol) promotes the ion currents caused by GABA, on the other hand at higher concentration it inhibits the action of GABA. Moreover, the sedative but not convulsive result in animals is triggered by the low-dose effect, whereas the large doses in insect herbivores act as insecticides [40].

The three polyacetylenes MS-1, MS-2, and MS-4 were obtained from *Cussonia zimmermannii* with recorded GABA(A) modifying activity [41].

However, the potency and/or affinity were demonstrated in the small micro molar range, but that varies significantly in terms of toxicity. Two structural characteristics (groups of allyl and terminal hydroxyl) that are present in five (most) poisonous natural products produced toxicity. It suggests that the terminal hydroxyl class is vital for the toxicity. Further, both the oenanthotoxins and dihydrooenanthotoxins require the allyl hydroxyl group but are highly toxic. On the other hand falcarinol and falcarindiol, which have an allylic class but not the final hydroxy group, showed decreased toxicity. None of the two "toxic characteristics" are present in the last three polyacetylenes group and are also not documented to display inhibitory behavior consistent with this theory. It would be necessary to investigate whether hydrolyzation has led to GABA (A) receptor antagonism because MS-4 has a terminal acetyloxy-group [38, 52].

4.3 Flavones

The substance Oroxylin A, allosterically to block GABA-mediated receptor by its action on chloride currents, and thus it describes the results of a previous in vivo study in which the substance exhibited antagonistic diazepam-induced effects [42, 43].

Wogonin was considered for the induction of GABA-induced chloride currents by using electrophysiological methods where it shown a stimulation of 57% at a concentration of 30 μM in the presence of 1 μM GABA where at 3 μM half maximal stimulation was noticed. It was also tested pharmacologically at a dose of 7.5, 15 and

30 mg/kg by using Elevated plus maze and hole board test. The wogonin showed anxiolytic effects. These data recommend that wogonin yielded anxiolytic by positive allosteric modulation of the $GABA_A$ receptor complex through benzodiazepine site interaction [43].

The chrysin is from *Scutellaria baicalensis* class which was separated from *Passiflora caerulea* [44, 63]. Chrysin was testified as partial agonist of central benzodiazepine receptors which reduced anxiety and does not induced sedative and muscle relaxation. The pharmacological effect of chrysin was observed in mice at 1 mg/kg in Elevated plus maze test. The anxiolytic effect was observed by increasing the number of entries and time spent in the open arm. The horizontal wire test showed a decreased percentage of animals grasping the horizontal wire, while in the hole board test an increase in time spent head-dipping at 3 mg/kg was observed, but no sedative effects at doses of 3 and 6 mg/kg [44].

Flavone compounds like wogoninnn and chrysin shows diazepam like anxiolytic effect whereas Oroxylin A antagonizing the effects provoked by diazepam.

4.4 Flavanes

(S) naringenin was isolated from the ethanol extract of *leaves Mentha aquatica* and evaluated against [^{3}H] flumazenil which exhibits an IC50 value of 26 mM. This compound can effectively modulate GABA function [45].

Glabrol, is the prenylated flavanone its three Diels-Alder type derivatives, sanggenon C, D, and G and were obtained from the root extract of *Morusalba*. All three molecules, with EC50 values in the range from 13.4 to 16.7 μM, increased chloride-induced GABA by over 700 percent (100 μM) [46].

In particular, two 8-lavandulyl-flavanones produced GABA-induced chloride impulses to potentiate by about 600 percent compared to the third 8-lavandulyl-flavanonol which is substantially less active.

The compounds like (S) naringenin, glabrol and 8-lavandulyl-flavanones acts at benzodiazepine site of GABA receptor which was analyzed using radio ligand binding assay.

4.5 Isoflavanoids and chalcones

Adenocarpus cinncinatus considered as the source of 2′, 4′, 7-trihydroxy-8-(3-methylbut-2-en-1-yl) isoflavone. Its stimulatory effect exhibits a uplift of GABA-induced chloride currents [64]. At a concentration of 30 nM, the substance increased GABA-induced chloride currents by 135 percent with a maximum potentiation of 581 percent at a level of 100 μM.

Isoliquiritigenin increased GABA-induced currents by of 151% at a dose of 10 M with a patch-clamp method on dorsal raphe neurons [48].

The *Sophora flavescens lavandulyl chalcone* is Kuraridine, which potentiates GABA-induced chloride currents by 719.7 percent at a dose of 10 M with a maximal activation rate of 891.5 percent [56].

The findings for isoflavonoids and chalcones are consistent with the results of the last two sections: isoflavone genistein blocks chloride currents in the same way as its flavone equivalents apigenin. The binding of [^{3}H] flunitrazepam inhibits chalcone isoliquiritigenin, furthermore the prenylated types show a marked ability of more than 500 percent (95.97) to around 900 percent.

In these compounds the substitution of one hydroxy and one methoxy group in both aromatic rings shows better potency. Overall, all of these compounds shown GABA (A) receptor agonist type action.

4.6 Terpenes

4.6.1 Monoterpenes

(+) borneol, (−) borneol, (−) bornyl acetate, is borneol, and camphor acting on GABA(A) receptors which were stated in *oocytes of Xenopus laevis*. With the increased stimulation reported for (+)-borneol and (−)-borneol, all other substances resulted in a marked maximum potentiation of GABA-induced chloride currents. EC50 values were, however, in the large micromolar range with the smallest score reported for bornyl acetate (111.2 µM) [49, 65].

In a radioligand binding assay measured on α and β thujone against [^{3}H] EBOB, where the substances displayed IC50 values of 13 and 37 µM. The β-thujone was identified as a non-competitive antagonist with an IC50 value of 21 µM in additional electrophysiological studies. Studies have confirmed these molecules acts by allosteric decrease of GABA-induced chloride currents. α-thujone has been reported in a survey on GABAergic miniature inhibitory currents to decrease their frequency and amplitude and to moderately influence their kinetics. The study concluded that alpha-thujone had gating receptor activity as this substance decreased the amplitude of current reactions to exogenous GABA and influenced their initiation, desensitization, and neutralization [50]. Epoxy-carvone was studied using MES, PTZ, and picrotoxin-induced seizure models for its anticonvulsant properties [66].

In *Xenopus oocytes*, thymol an aromatic monoterpene is known from a variety of Thymus species, was examined on $\alpha_1 \beta_2 \gamma_3$ where chloride-induced GABA-currents increased by 416% at a concentration of 100 µM [51].

Isopulegol has been tested in-vivo for its anxiolytic ability. In the hole board test and Elevated plus maze [EPM] test at a concentration of 25 and 50 mg/kg, the isopulegol has been shown to raise the number of head dips in the hole board test which specifies anxiolytic effect in which the number of open arm entries along with the time spent in the arm was also increased in EPM test. In the EPM test of isopulegol results reduced the animal's aversion to the open arms as well as promoted the exploration which specifies anxiolytic effect [67].

In an anxiolytic-like behavioral study, the (+)-limonene epoxide at various doses of 25, 50, and 75 mg/kg showed an improvement in open arms inputs and time spent in open arms in the EPM test and decrease in the number of crossing, grooming, and rearing is found in the open field test, further implying the sedative effects of the drug [55]. The anxiolytic effect was reported by a follow-up study in which the compound demonstrated a decrease in the number of buried marbles in the buried marble test at a dose of 25, 50, and 75 mg/kg [68]. In several studies, Carvacryl acetate was also tested for anxiolytic and sedative effects. The EPM test shows that the compound increased the number of open arm entries at a dose of 100 mg/kg and the time spent in the open arm at doses from 25 to 100 mg/kg. In case of the light/dark test it increased the number and time spent in the light area at doses from 25 to 100 mg/kg. In the buried marbles test reduction of buried marbles number was observed at doses from 25 to 100 mg/kg, but no co-ordination impairment in the Rotarod test and no decrease in locomotor activity is observed in the open field test were measured at the same doses [69].

A few monoterpenes have been studied for their GABA receptor modulation action and the highest potential of chloride channel opening was observed for bicyclic alocohols, like (+) and (−)-borneol whereas isoborneol showed distinct potentiation. Oxidation of the hydroxy-group or the presence of an exocyclic methylene group causes decrease in the activity. The only monocyclic monoterpenes positive receptor modulation was observed by thymol.

4.6.2 Sesquiterpenes

Two monoterpenoid moieties namely α caryophyllene and β caryophyllene belonging to *Sideritis sp*. that displayed medium modulation of GABA-mediated chloride channels (117 and 115%, respectively).

Curdione and curcumol were extracted from the oil of *Curcumaerhizoma* and were tested on GABA$_A$ receptors expressed. The molecules increased GABA-mediated chloride channel activity with 133 and 175.7%, respectively at a concentration of 50 μM. The EC50 value of Curcumol was found to be 34.4 μM and the highest activity of 251% was found at 300 μM [53].

The highest induction of GABA-mediated chloride channel of around 400% was found in (+) cuparenol and (+)-dihydrocuparenic acid.

At 300 μM, when Atractylenoids I, II and III from *Atractylodesmacrocephala* was tested on GABA (A) receptors highest stimulation of 96 to 166% was observed with an EC50 value of 12, 70 and 99 μM, respectively [54, 70].

Anisatin is oxygenated sesquiterpene lactone separated from *Illicium anisatum* is a potent noncompetitive antagonist of GABA$_A$ receptor that has an activity similar to picrotoxin [71]. Studies demonstrated that anisatin at 1 μM decreased chloride currents created by 30 μM GABA to 41.7%. The IC50 value was measured with 1.10 μM along with an IC50 value of 0.42 μM for picrotoxinin, which is the active compound of picrotoxin was obtained. An indication that anisatin binds to the picrostatin site of the receptor was shown in a radioligand binding assay with an IC50 value of 0.43 μM against [3H] EBOB. One very potent sesquiterpene is xenovulene A, which was separated from the fungus Acremonium striatum (now classified as Sarocladium striatum) [72].

As a result of the structural differences of the sesquiterpenes only restricted conclusions on their structure–activity relationship can be drawn. Reduction of the acidic function to an alcoholic function does not change the activity whereas the change of the isopropenyl-function of compound to a plane isopropanyl-moiety leads to a significant loss of activity.

4.6.3 Diterpenes

In this Section 14 diterpenes which are having the actions on GABA are discussed. Miltirone, a Salvia miltiorrhiza tanshinone, was assessed against [3H] flunitrazepam with an IC50 value of 0.3 M in a radioligand-binding analysis [73].

Dehydroabietic acid has been segregated and examined in *Xenopus laevisocytes* from *Boswellia thurifera*, now known as *Boswellia serrata* [47]. GABA-induced chloride currents were enhanced by the substance by 397.5 percent at 100 μM and displayed an EC50 value of 8.7 μM. Isopimaric and sandaropimaric acid were extracted and examined from *Biota Orientalis*, currently known as Platycladusorientalis, in the Xenopus oocyte assay [74]. The substances showed a maximum stimulation effect of 425.2 and 855.7 percent of GABA-induced chloride currents at 500 μM and EC50 values of 141.6 and 33.2 μM, respectively.

Two diterpenes of phyllocladane namely 17-dihydroxyphyllocladane-3-one and 16,17,18-trihydroxyphyllocladane-3-one types were obtained from *Aloysia virgata* and assessed for GABA(A) affinity to [3H] flumazenil with inhibitory constant [Ki] of 111 and 56 μM. Both compounds were studied in vivo, with compound, 17-dihydroxyphyllocladane-3-one which exhibits increased locomotor activity at a dose of 1 mg/kg in the locomotor activity test and increased rearing at 0.3 and 1 mg/kg in the hole board test. Compound 16,17,18-trihydroxyphyllocladane-3-one increased the number of head dips at 0.3 and 3 mg/kg, the number of rears at a dose of 1 mg/kg and the time spent head-dipping at a dose of 3 mg/kg. The compound at a dose

of 1 mg/kg increased the number of open arm entries in the EPM test and the time spent in the light area as well as the number of transitions in the light/dark test [75].

Two diterpenes of type labdane, cerumin A and coronarin D, were obtained from Curcuma kwangsiensis [57]. In the Xenopus oocyte assay, substances at a 300 M concentration stimulated GABA-induced chloride currents by 309.4 and 211.0 percent, with EC50 values of 24.9 and 35.7 M. Ginkgolides A B and C which are diterpene trilactones of *Ginkgo biloba*, are moderately active GABA$_A$ receptor antagonists with Ki values of 14.5, 12.7 and 16.3 M in *Xenopus laevis oocytes* [58].

Some results suggest that compounds like 7-methoxyrosmanol and galdosol increases 10-fold receptor affinity by an oxo-group at 7^{nth} position instead of methoxy group. On the other hand, for compounds isopimaric acid and sanda-ropimaric acid, the change from the 7th to the 8th position of the double bond and thus to the C-ring of the substance doubles the maximum stimulatory effects and significantly decreases the EC50 value There are no clear variations in the inhibitory action of bilobalide and ginkgolide A-C in their IC50 values or in their ability to inhibit chloride current induced by GABA. Therefore, all these diterpenes works as GABA receptor agonists which help in chloride current flow.

4.6.4 Triterpenes

Asiatic acid was separated from *Centella Asiatica* and its anxiolytic effects were analyzed in the EPM test. The compound displayed no action on the open arm time but reduced the motile time and the highest speed at 30 mg/kg. These actions were blocked by flumazenil [76].

Ginsenoside C, is a glycoside isolated from *Panax ginseng* was tested on GABA(A) receptors expressed in xenopus laevis oocytes which were found to potentiate GABA-induced chloride currents with an EC$_{50}$ value of 53.2 μM [77].

Four cycloartane glycosides actein, cimigenol-3-O-β-D-xylopyranoside 25-O-acetylcimigenol-3-O-α-L-arabinopyranoside, 23-O-acetylshengmanol-3-O-β D-xylopyranoside were extracted from *Actaea racemosa* root systems (black cohosh) and assessed in *Xenopus laevis* oocytes for their capacity for GABA-induced chloride currents [74]. Substances like actein, cimigenol-3-O-β-D-xylopyranoside and 25-O-acetylcimigenol-3-O-α-L-arabinopyranoside which are isolated constitu-ents from rhizomes of Actaea rasemosa exhibited potentiation of GABA-induced chloride currents in the range of 256 to 378 percent at a concentration of 300 M, while 23-O-acetylshengmanol-3-O-D-xylopyranosides reported stimulation of 1947 percent and were also shown to generate small chloride currents due to lack of GABA. The EC50 values for the four glycosides were estimated from 26 to 36 μM. The pentose moiety cleavage led to a substantial decline in anxiety-related behavior (particularly for substance 23-O-acetylshengmanol-3-O-D-xylopyranosides. This compound was used in several in-vivo studies for the examination of its anxiolytic and sedative properties. It increased the number of open entries at 0.6 mg/kg in the EPM test whereas reduced stress-induced hyperthermia at doses of 0.2, 0.6, 2 and 6 mg/kg. In the open field test, this compound reduced the distance traveled at doses of 6, 20 and 60 mg/kg and also increased the time spent in the centre at a dose of 60 mg/kg, while the number of entries into the centre was reduced [60].

The discussion of the structure–activity of triterpenes is not influenced by the lack of comparable structures (scaffolds) compared to the last two subsections, but by the variety of test systems used for their analysis. However, it is possible to compare at least some of the known triterpenes from ginseng and black cohosh. Electrophysiological data showed lower EC50 levels for the three ginseng triter-penes ginsenoside C. Unfortunately, the maximum chloride current stimulation

values were only observed for the two aglycones and were recorded to be 54.1 and 23.3 percent, respectively (at a concentration of 100 M). It can be concluded that the receptor modulation of the glycoside would be of significant concern after examining substance 23-O-acetylshengmanol-3-O-D-xylopyranosides, where the xylose moiety cleavage changed the potentiation of GABA-induced chloride currents from 1692 percent to 64 percent (100 M) and thus into the range of ginseng aglycon. Both compounds 23-O-acetylshengmanol-3-O-D-xylopyranosides and ginsenoside C disclose a four-ring structure with a side chain linked to ring D when contrasting their scaffolds. The prenylate and oxyprenylate side chains have enhanced activity, which is reminiscent of the structure-action-relationship of coumarins. The ginsenoside side-chain will stand for the prenyl moiety in the case of the triterpenes under consideration and that of substance23-O-acetylshengmanol-3-O-D-xylopyranosides for the more active epoxylated form. However, this molecule has additional characteristics that may contribute to its pronounced effect, such as keto-function at position 16 or acetyloxy-group at C-23, which both differentiate the compound from the other slightly less active cycloartanoids [59].

The neurosteroid binding site would be most obvious and consistent with the fact that neurosteroids are the most effective natural GABA$_A$ receptor modulators and, in the absence of GABA, are also capable of evoking chloride currents [78]. However, the hydroxy group at position 3 and the keto group at position 17 or 20 are considered to be important for neurosteroid binding activity. As far as the structure of compound is concerned, the keto group may well lead to the binding of the receptor in position 16 instead of position 17, but the fact that the role of the compound almost vanishes with the xylose moiety does not support this theory unless the binding of the neurosteroid site can be improved by the residue of sugar instead of the hydroxy group in position 3. Barbiturates, on the other hand, are also known to activate GABA(A) receptors directly at higher concentrations and the site of barbiturate binding is thought to be similar to that of neurosteroids [79].

5. Conclusion

Natural products with GABA receptors activity were identified in the literatures and discussed in this chapter. Depending on the number of related compounds and test systems used, it was possible to draw in the vicinity of conclusions regarding their structure–activity relationships. As most of the studies examined flavones, and these studies mainly applied radio ligand binding assays, substitution patterns responsible for increased receptor affinity could be associated with one flavone even with diazepam-like Ki values. As far as receptor regulation is concerned, flavones are either non-competitive antagonists or partial agonists. However, certain compounds also exhibited anxiolytic or anticonvulsant effects. Other phenolic compounds addressed in this study were, for example, coumarins, where prenylated compounds demonstrated higher stimulation of the receptor. The association of phenyl residues and pronounced receptor modulation has also been observed for flavanes, isoflavonoids, and chalcones and may be of interest to the production of GABA(A) receptor modulators. Besides, the structural features required for the positive or negative alteration of the polyacetylene and monoterpene receptors as well as the effect of deglycosylation on certain triterpenes have been highlighted. Very few studies have been found on the subtype-specificity of natural products. One example is the enhanced modulation of isopimar and sandaropimaric acid receptors after the exchange of α1-subunit for α2 or α3-subunits. Neolignane honokiol must also be stated in this sense, although the effect was more dependent

on the GABA(A) receptor subunits. Data obtained from recorded in vivo studies may be helpful in this regard, as many compounds have been known to exhibit anxiolytic effects without exhibiting sedative or muscle relaxant properties.

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